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## **Relation between seal integrity and hygienic quality in silage bales and differences between baling techniques**

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### **Introduction**

Bale silage is widely used in many countries on small and medium sized cattle farms and for horses. About 8 million bales are produced annually in Sweden, corresponding to approximately 45% of the total silage production of 4.5 million ton DM (Pettersson, 2006; SCB, 2016). Sufficient protection against air intrusion during storage is essential in all ensiling, and since introduction of the round bale technique, seal integrity of bales has continuously been improved. When the technique to make silage in bales was introduced, bales were inserted in plastic bags but this was soon replaced by the stretch film technique. During the last two decades, the polyethylene industry has continuously developed new stretch film qualities which have been tested on round and square bales of grass at the Department of Animal Nutrition and Management, SLU. Degree of air-tightness of bales, seal integrity have been measured in these studies by the time it takes for an induced under-pressure to disappear in the bales. Silage quality has thereafter been analyzed in terms of standard chemical analysis of fermentation parameters such as pH, ammonia nitrogen and volatile fatty acids and hygienic standard in terms of cell counts of yeast and mould.

The main purpose of the present work was to evaluate the relation between seal integrity, measured as pressure equalization time, and fermentation parameters and hygienic quality. Furthermore, data was used in a meta-analysis to study how silage quality was affected by number of stretch film layers and by using round- or square bales.

### **Materials and Methods**

Data consisted of 29 experiments where seal integrity and silage quality parameters were analysed. Square bales had been used in 7 of the 29 experiments, while round bales were used in the remaining experiments. Crops were pure grass or grass/clover leys where clover proportion varied from 5 to 40% and had been harvested in first or second harvests in the southern half of Sweden (55° to 59°N). Bales were made with 4, 6 or 8 layers of 750 mm wide stretch film with a thickness from 17-25 µm. The plastic was in most cases applied on netted bales but in some experiments, mantel film was used (Spörndly & Nylund, 2016). White stretch films were most common but, occasionally, some light green films were used and in two experiments, black films were used. Combined machines for pressing and wrapping were used in all experiments. All treatments were made with 6 replicate bales and in many experiments, several stretch films with different chemical compositions were tested for company product development purposes. Data comprised analyses of 1193 bales.

Bales were stored for at least 100 days after which they were subjected to seal integrity measurements where an under-pressure of -200 Pa was applied via a non-return valve. Level of air tightness was determined by time in seconds for under-pressure to be reduced to -150 Pa by air penetrating the seal. In the process of gas evacuation, carbon dioxide content (% of volume) in the bales was measured with a portable gas analyser GA 2000 (Geotechnical Instruments, Warwickshire, UK). After removing the stretch film, visible spots of yeast and

mould on bale surfaces were measured and areas of visible yeast or mould was expressed as percent of total bale surface area. Six cores (35 mm wide, 700 mm deep) per bale were then drilled and pooled into one sample per bale for chemical analyses. Dry matter (DM) and water soluble carbohydrates (WSC) were analysed on the pooled samples and pH, ammonia nitrogen (NH<sub>3</sub>-N), ethanol, 2,3-butanediol, lactic, acetic, butyric and succinic acid were analysed on the liquid phase. All analyses were performed with wet chemistry methods as described by Åkerlind et al. (2011).

Correlation calculations between seal integrity (s) and surface yeast and moulds, CO<sub>2</sub> content and chemical fermentation parameters were performed using PROC CORR statement (SAS, 2014). Statement PROC GLM was used to investigate effects of bale type and number of stretch film layers. In all statistical calculations, bale was considered an experimental unit and effects were considered as statistically significant when  $P < 0.05$ .

## Results and Discussion

Seal integrity was negatively correlated with yeast and mould on bale surface and positively correlated to carbon dioxide concentration and to lactic acid, ammonia nitrogen and succinic acid content in silage (Table 1). Correlations were low but still significantly different from zero. This implies that the method for measuring seal integrity works. A better seal integrity gives a better barrier between the atmosphere rich in carbon dioxide inside the bale and the outside air, resulting in less visible mould growing on the bale surface. The magnitude of the correlation was as mentioned lower than expected. It could be due to the variable dry matter content of bales in the different experiments, varying from 22 to 80%. Dry matter content is the main factor determining fermentation processes and this was not accounted for by simple correlation coefficients.

**Table 1** Pearson correlation coefficients (r) of seal integrity and dry matter content with visible yeast and mould on bales, carbon dioxide content and a number of silage fermentation characteristics

| <i>Seal integrity</i> |       |       |                 |      |       |       |             |             |              |               |         |               |
|-----------------------|-------|-------|-----------------|------|-------|-------|-------------|-------------|--------------|---------------|---------|---------------|
|                       | Yeast | Mould | CO <sub>2</sub> | DM   | pH    | Amm-N | Lactic acid | Acetic acid | Butyric acid | Succinic acid | Ethanol | 2,3-butandiol |
| <i>r</i>              | -0.07 | -0.13 | 0.32            | 0.02 | -0.03 | 0.08  | 0.10        | 0.02        | 0.02         | 0.30          | -0.05   | 0.08          |
| <i>Sign.</i>          | *     | ***   | ***             | Ns   | Ns    | *     | **          | Ns          | Ns           | ***           | Ns      | Ns            |
| <i>N</i>              | 1182  | 1180  | 490             | 1183 | 1183  | 698   | 754         | 692         | 330          | 243           | 646     | 543           |

  

| <i>Dry matter content</i> |       |       |                 |                |      |       |             |             |              |               |         |               |
|---------------------------|-------|-------|-----------------|----------------|------|-------|-------------|-------------|--------------|---------------|---------|---------------|
|                           | Yeast | Mould | CO <sub>2</sub> | Seal integrity | pH   | Amm-N | Lactic acid | Acetic acid | Butyric acid | Succinic acid | Ethanol | 2,3-butandiol |
| <i>r</i>                  | 0.03  | 0.07  | -0.27           | 0.02           | 0.77 | -0.82 | -0.55       | -0.53       | -0.45        | -0.44         | -0.17   | -0.46         |
| <i>Sign.</i>              | Ns    | *     | ***             | Ns             | ***  | ***   | ***         | ***         | ***          | ***           | ***     | ***           |
| <i>N</i>                  | 1192  | 1190  | 490             | 1183           | 1193 | 703   | 761         | 699         | 335          | 243           | 651     | 545           |

N=number of observations. Ns= $p > 0.05$ . \* =  $p < 0.05$ . \*\*= $p < 0.01$ . \*\*\* $p < 0.001$ .

Correlations between DM content and silage characteristics are shown separately in Table 1. The dominating influence of dry matter content was probably the main reason for the low correlation between seal integrity and effects on silage.

Analysis of effect of bale type and number of stretch film layers are in Table 2. Compared to square bales, round bales were better sealed with more than three times longer equalization times. Number of stretch film layers also had a clear effect on seal integrity. The major improvement took place when the number of layers increased from 4 to 6 layers, whereas further increase in air-tightness from 6 to 8 layers was considerably less. Increasing layers and a better seal integrity was also reflected in a higher carbon dioxide content in the bales and less mould growing on the surface.

**Table 2** Effect of bale type and number of bale stretch film layers on seal integrity, carbon dioxide content and areas of yeast and mould on bale surface. N= number of observations

|                                 | <i>Effect of bale type</i> |                   |                   |       | <i>Effect of stretch film layers</i> |                   |                   |                   |       |
|---------------------------------|----------------------------|-------------------|-------------------|-------|--------------------------------------|-------------------|-------------------|-------------------|-------|
|                                 | N                          | Square            | Round             | p<    | N                                    | 4                 | 6                 | 8                 | p<    |
| <i>Seal integrity, sec</i>      | 1183                       | 149 <sup>a</sup>  | 547 <sup>b</sup>  | 0.001 | 1183                                 | 112 <sup>a</sup>  | 410 <sup>b</sup>  | 564 <sup>c</sup>  | 0.001 |
| <i>CO<sub>2</sub>, %</i>        | 490                        | 56.8 <sup>a</sup> | 43.2 <sup>b</sup> | 0.001 | 490                                  | 40.1 <sup>a</sup> | 57.6 <sup>b</sup> | 69.9 <sup>c</sup> | 0.001 |
| <i>Yeast, % of bale surface</i> | 1192                       | 0.05 <sup>a</sup> | 0.09 <sup>b</sup> | 0.048 | 1192                                 | 0.11              | 0.07              | 0.04              | 0.211 |
| <i>Mould, % of bale surface</i> | 1190                       | 1.88              | 1.98              | 0.604 | 1183                                 | 1.11 <sup>a</sup> | 0.44 <sup>b</sup> | 0.17 <sup>b</sup> | 0.001 |

Superscripts (a, b, c) on the same row within effect indicate significant difference at  $p < 0.05$ .

The better seal integrity of round bales compared to square bales was not reflected in higher carbon dioxide content or less yeast and mould growth. For carbon dioxide, this can possibly be explained by the fact that carbon dioxide measurements were introduced relatively late in the series of experiments and were, therefore, only measured in one of the seven experiments with square bales. This was, however, not the case for yeast and mould measurements but for mould, there was no difference and for yeast, the effect was barely significant ( $P=0.048$ ).

## Conclusions

The method for measuring seal integrity of silage bales by pressure equalization time was positively correlated to measurements reflecting other signs of seal integrity, such as bale carbon dioxide concentration. It was also shown that seal integrity measured in this way was associated with growth of yeast, and particularly, growth of mould. The meta-analysis of 29 experiments clearly showed that more layers of stretch film resulted in bales with a better protection from air intrusion and growth of mould. Improvements were greater when increasing from 4 to 6 layers than when increasing from 6 to 8 layers.

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